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ADDENDUM



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ABSTRACT

Millions of children worldwide are born with rare and debilitating developmental disorders each year. Although an increasing number of these conditions are being recognized at the molecular level, the characterization of the underlying pathophysiology remains a grand challenge. This is often due to the lack of appropriate patient material or relevant animal models. Dogs are coming to the rescue as physiologically relevant large animal models. Hundreds of spontaneous genetic conditions have been described in dogs, most with close counterparts to human rare disorders. Our recent examples include the canine models of human Caffey (*SLC37A2*), van den Ende-Gupta (*SCARF2*) and Raine (*FAM20C*) syndromes. These studies demonstrate the pathophysiological similarity of human and canine syndromes, and suggest that joint efforts to characterize both human and canine rare diseases could provide additional benefits to the advancement of the field of rare diseases. Besides revealing new candidate genes, canine models allow access to experimental resources such as cells, tissues and even live animals for research and intervention purposes.

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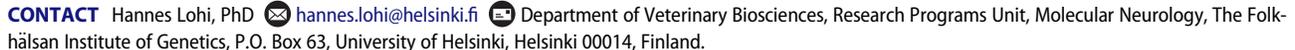
Caffey; dog; disease; gene;
Raine; van den Ende-Gupta

Introduction

Dog, *Canis lupus familiaris*, is considered to be the first domesticated animal. Well-supported evidence suggests that all dog breeds originate from the Eurasian gray wolf (*Canis lupus lupus*), potentially from Southeast Asia,¹ although the exact geographical location and the timing of the process remains elusive.² The majority of the 400 current breeds, each defined by specific physical and behavioral characteristics, are less than 200 y old. The phenotypic variation seen in dogs today is mainly a result of recent strong artificial selection driven by dog breeders admiring distinctive features. The establishment of registering bodies (e.g. kennel clubs), together with breeding standards, has led to controlled and restricted breeding practices. Many breeds have arisen from a limited number of founder animals and the use of popular sires is a common practice. As a consequence, each breed represents an isolated breeding population with high levels of phenotypic

homogeneity, reduced genetic diversity and enrichment of breed-specific disorders.

The sequencing of the canine genome and subsequent development of powerful gene discovery tools has remarkably facilitated gene discoveries in disease, morphology and behavior.³⁻⁵ Dogs possess a unique genetic architecture, consisting of an ancient short and recent long-range linkage disequilibrium (LD).^{3,6} This genetic architecture facilitates gene discovery by providing a powerful mapping approach within a breed while an across-breed mapping may significantly narrow down the associated region in order to expedite the identification of the causal risk variant as efficiently exemplified in a study of canine ectodermal dysplasia.⁷ The dog genome is less divergent from the human genome than from the mouse genome and, therefore, more of the human genomic sequence can be syntetically aligned to the genome of the dog than that of the mouse.³ Dogs have approximately the same number of genes as humans, most of them being close orthologues.

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Dog is a large animal model and physiologically and clinically more similar to human than mouse. Importantly, pet dogs share also the environmental conditions of their owners and are thus not only affected by genetic traits but also by “life style.” Hundreds of spontaneously occurring common canine conditions are analogous to human diseases such as diabetes, cancers, epilepsies, eye diseases and autoimmune diseases, not to mention the high numbers of rare monogenic diseases. Canine heritable monogenic diseases, with the current list of 201 known causative genetic variants, are recorded in the public database Online Mendelian Inheritance in Animals (OMIA) (<http://omia.angis.org.au>). Most of the genes underlying canine diseases are orthologues of the genes in the corresponding human rare conditions.

Well-recorded genealogical data is also available, in many places, for dogs, which can assist in the design of genetic studies. Purebred dog registries are maintained by kennel clubs or other equivalent organizations. For example, the Finnish Kennel Club maintains an open dog registry in Finland (<http://jalostus.kennelliitto.fi>) and contains useful information about each registered Finnish dog, *e.g.*, pedigrees and some results of health examinations. Dogs also have

good-quality veterinary medical care available and professional veterinary records are essential to reliable and successful research.

To utilize the canine models of human hereditary disorders, we have established a comparative genetic program in Finland with a large canine biobank, including ~60 000 samples from over 300 breeds of dogs. Leveraging this biobank, the unique canine genetic architecture and genomic tools, the presence of hundreds of breed-specific conditions, and active research participation of the canine and veterinary communities, we have reported numerous new gene discoveries in dogs across disease groups,⁷⁻¹³ including several developmental disorders that model human pediatric disorders. The identification of the genetic causes establishes the affected breeds as pre-clinical models, including examples such as the recombinant protein replacement for X-linked hypohidrotic ectodermal dysplasia (XLHED),¹⁴ and gene therapies for hemophilia A¹⁵ and retinal degeneration.¹⁶ Given the accelerating pace of gene discoveries, new opportunities exist for intervention trials. A summary of the special features that facilitate the use of the dog as a complementary model to human disorders is shown in Fig. 1.

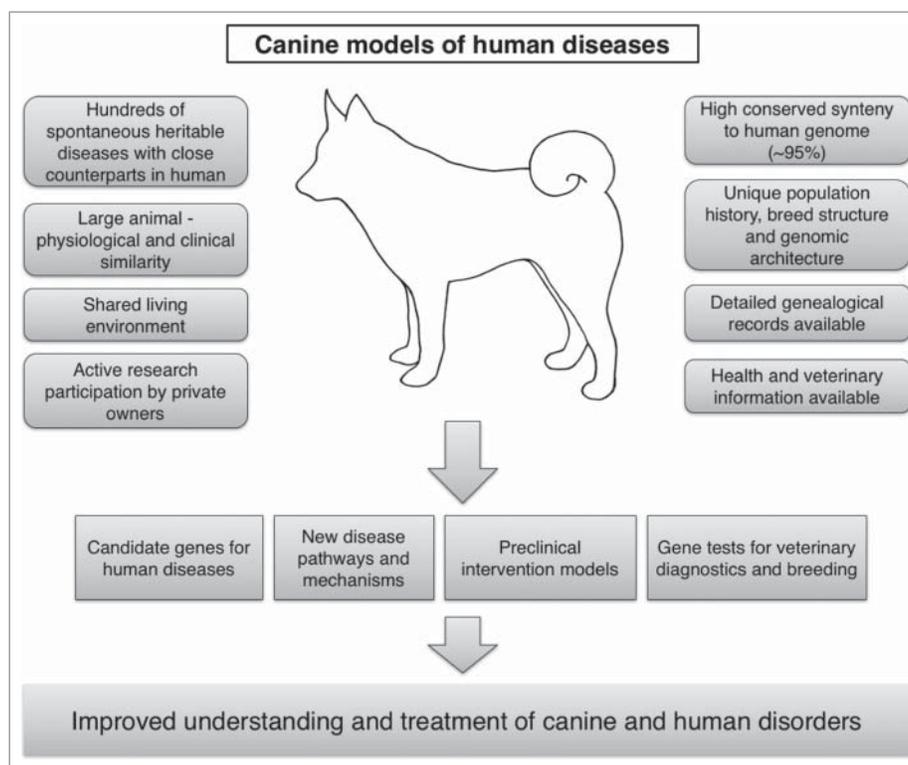


Figure 1. Various clinical, genetic, physiological and environmental characteristics in dogs make them excellent comparative models for facilitating the understanding and treatment of human disorders.

Our most recent study revealed 3 new developmental disorders and causative genes in dogs.¹⁷ We identified *SLC37A2* as novel candidate gene for human infantile cortical hyperostosis (Caffey disease) and implicated *SCARF2* and *FAM20C* variants in the canine forms of van den Ende-Gupta and Raine syndromes, respectively. The study demonstrates the power of next-generation sequencing approaches in identifying the causative genes, the molecular diagnoses and the names of illnesses in canine syndromes with high relevance to rare human diseases. All 3 spontaneous canine conditions closely resemble the human conditions and provide physiologically relevant models to better understand poorly characterized gene functions and molecular pathologies toward new therapeutic innovations.

A novel sugar-phosphate transporter candidate gene for infantile hyperostosis

Cranio-mandibular osteopathy (CMO) is a self-limiting hyperostosis with a painful swelling of the jaw, leading to dysphagia and difficulty in opening the mouth in young dogs. It is equivalent to human infantile cortical hyperostosis.^{18,19} We discovered a splicing defect in the *SLC37A2* gene in CMO and propose it as an excellent candidate for mutation screening in Caffey patients. Our discovery is important since the molecular etiology remains unknown in many cases and early molecular diagnosis would avoid invasive procedures. Furthermore, it is still mechanistically unclear how the previously-discovered missense variant in *COL1A1* could lead to self-limiting hyperostosis.^{20,21} *SLC37A2* may provide new insights to disease etiology.

SLC37A2 belongs to the *SLC37* family of 4 ER-associated glucose-phosphate transporters.²² It is abundantly expressed in disease-relevant tissues such as bone marrow and haematopoietic cell lineages such as osteoclasts and macrophages,^{23,24} and may play a central role in glucose homeostasis in osteogenesis. We hypothesize that an impaired function of *SLC37A2* disturbs proper glucose supply in the osteoclasts, decreasing their overall activity, which in turn results in an imbalance between osteoblastic and osteoclastic functions in the developing bones leading to hyperostosis. Interestingly, *SLC37A2* has been found also as a primary target for vitamin D,²⁵ which could be a potent therapeutic booster to alleviate clinical signs in

affected dogs, which are due to diminished expression of wild type expression of *SLC37A2*.

Our canine study not only discovered a novel Caffey disease gene but also unraveled a physiological function of *SLC37A2*, providing new insights and hypotheses into infantile swelling diseases, which may be related to impaired glucose homeostasis during the bone development. The affected dogs now provide unique resources to address these hypotheses and other *SLC37A2*-related mechanisms in osteogenesis biology.

A canine model of van den Ende-Gupta syndrome

Our second example highlights the importance of the gene discovery to naming the illness and establishing a model for the human syndrome. We found a severe truncating mutation in *SCARF2* in an unknown developmental syndrome in Wire Fox Terriers, characterized by severe mandibular prognathia and other skeletal abnormalities. The *SCARF2* mutations have been found in less than 30 reported patients of van den Ende-Gupta syndrome (VDEGS). These patients are characterized by craniofacial and skeletal abnormalities including blepharophimosis, a flat and wide nasal bridge, narrow and beaked nose, hypoplastic maxilla with or without cleft palate and everted lower lip, prominent deformed ears, down-slanting eyes, arachnodactyly and camptodactyly.²⁶

Human and canine VDEGS patients share many similarities, including hypoplastic maxilla, dislocated radial head, patellar dislocation, deviated nasal septum and small eyes.¹⁷ Therefore, the *SCARF2* discovery established the canine diagnosis and a model for human VDEGS. This is important since the lack of a transgenic mouse model and scarcity of human patients highlight the role of affected dogs as a novel resource for understanding *SCARF2* functions and molecular pathology. In addition, given that some of the VDEGS dogs survive past 10 years, they could potentially serve as preclinical models.

SCARF2 is a very poorly-characterized member of the scavenger receptor type F family.²⁷ It is a single-pass transmembrane protein with homology to calmodulin (CaM)-like Ca^{2+} -binding proteins. The extracellular domain contains several putative epidermal growth factor-like (EGF) domains, and it has a number of positively-charged residues within the intracellular domain. *Scarf2* is expressed in disease-

relevant tissues such as epidermis, branchial arches, mandible, maxilla and urogenital ridge tissue of developing mouse embryos.^{28,29} However, nothing is known about its function.

A detailed histopathological characterization of the canine VDEGS should be performed to learn about *SCARF2* functions, regulation and the related cellular pathway, in order to improve our understanding of the disease mechanisms, which in turn could lead to therapeutic hypotheses. The established canine model provides powerful new resources toward these aims.

A canine model of Raine syndrome

FAM20C mutations have been described in human autosomal recessive osteosclerotic bone dysplasia (Raine syndrome). Less than 40 cases with variable severity and clinical heterogeneity have been reported including those which survive into childhood.³⁰⁻³³ Typical characteristics include variable extent of hypophosphatemia, abnormal and hypomineralized teeth, craniofacial anomalies, such as exophthalmos, midface hypoplasia, microcephaly and cleft palate, as well as gingival hyperplasia, generalized osteosclerosis and intracerebral calcifications.

We found a recessive missense variant in the kinase domain of the *FAM20C* gene in a family of several affected dogs that suffered from severe dental wear and loss of teeth. Clinical findings in dogs were limited to severe hypomineralization of teeth, leading to extensive wear and inflammation as prominent features. We did not observe some of the typical gross changes described in Raine patients such as hypophosphatemia and craniofacial anomalies. Our canine study raises the question of whether a more limited human phenotype exists and should be targeted for mutation screening. However, there is a significant clinical heterogeneity in the symptoms between human patients and more detailed radiographic analyses should be performed in dogs to observe potential mild changes outside the dental phenotype.

Different species may expand our understanding of the heterogeneity of the clinical picture and provide important information about genotype-phenotype correlations. *Fam20c*-deficient mice have a prominent dental phenotype.³⁴ Our *FAM20C*-deficient dogs have a dental phenotype similar to that of mice and humans. Considering that, unlike rodents, dogs have a dental physiology similar to that of humans, having

both deciduous and permanent dentitions, they provide a relevant preclinical model for this rare human bone disease.

Conclusion

We and others have demonstrated that dogs carry numerous clinically and genetically close counterparts of human rare diseases. Better characterization and utilization of the canine models could be highly useful, not only for the development of new therapies for rare human diseases, but also for the better understanding of the molecular pathophysiology of the conditions in general. A rapidly-growing number of new gene discoveries in dogs will establish interesting new models for human disease. Direct encouragement of private pet owners to participate in preclinical trials could provide a straightforward route to facilitate the validation of human treatment approaches while also benefitting canine health and welfare. The full preclinical potential of canine models has not been realized yet.

Disclosure of potential conflicts of interest

HL is an owner of Genoscooper Ltd., a DNA diagnostics company that provides genetic testing for dogs.

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